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TITLE: Siah1/2 Ubiquitin Ligases in ER Stress Signaling in Melanoma

PRINCIPAL INVESTIGATOR: Ze'ev Ronai

RECIPIENT: Sanford-Burnham Medical Research Institute
La Jolla, CA 92037

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Fort Detrick, Maryland 21702-5012

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6. AUTHOR(S)
Ze'ev Ronai, PhD. Professor

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Sanford-Burnham Medical
Research Institute
10901 North Torrey Pines Rd
La Jolla, CA 92037

8. PERFORMING ORGANIZATION REPORT NUMBER

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U.S. Army Medical Research and Materiel Command
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13. SUPPLEMENTARY NOTES

14. ABSTRACT

The task of identifying novel and significant ER stress related changes that are regulated by ubiquitin ligases was initiated with a focus on Siah1/2, which led to a distinct understanding of these ligases' impact on melanoma, a study which continues. We have also identified the ubiquitin ligase RNF5, as one that regulates the immune checkpoint control mechanism, an ER stress associated ligase. We continue with exciting and significant studies as we define unexpected ER-stress related roles for the ubiquitin ligase RNF5 in melanoma. Lastly, our unbiased search for ubiquitin ligases that may affect melanoma resistance to BRAF inhibitor therapy led us to identify and characterize RNF125, which is downregulated in resistant tumors with concomitant increase in JAK1 and receptor tyrosine kinases. These important findings were published in *Cell Reports* (2015) and pave a path for new clinical trial for patients with resistant melanoma to BRAF inhibitors. We expect that the work with our current subset of Siah1/2 inhibitors will be summarized for publication during 2016/7.

15. SUBJECT TERMS

Siah1, Siah2, UPR, UBL, RNF5, RNF125, ubiquitin ligases, ER stress, JAK1, EGFR, melanoma, BRAF inhibitor resistance

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	15	19b. TELEPHONE NUMBER *(include area code)*

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1. INTRODUCTION

The finding that Siah1/2 ubiquitin ligases expression may define a select cluster of melanoma led us to explore the precise role of each of these ubiquitin ligases in melanoma. Our studies allow for the first time to map the network of Siah1 and Siah2 regulated genes, revealing for the first time that these two family members represent distinct networks and are associated with different melanoma tumors. Given the role of Siah1/2 in control of the UPR and ER stress, as well as in the control of hypoxia, understanding mechanisms underlying each of these two ligases activity in melanoma is of crucial importance and will be continued during the second year of this funding. Parallel work on the ER stress associated ubiquitin ligase RNF5 (component of the ER associated degradation), led us to discover that it is directly involved in the regulation of immune checkpoint pathways. The significance of this finding is reflected in the limited growth (~30–40% of what is seen in wild type (WT) animals) of aggressive melanoma (Braf/Pten/Cdkn2a) when inoculated in RNF5 knockout (KO) animals. Significant increase in CD4 and CD8 positive T cells in the melanoma grown in RNF5 KO mice support a major role for this ubiquitin ligase on immune checkpoint control. In addition, we performed an unbiased screen to identify ubiquitin ligases that may be involved in the regulation of melanoma resistance to vemurafenib, a major obstacle in clinical management of melanoma today. Our search led to identify RNF125, which we found to regulate JAK1 stability with concomitant effect on EGFR and other receptor tyrosine kinases. The immediate implication of the funding of this project pertains to the possibility that JAK1 inhibitors may be used in clinical trials to defeat the resistance of such melanomas, an aspect that is currently being evaluated. Common to all 3 ubiquitin ligases is their intimate tie with ER stress and the direct implications to distinct aspects of melanoma development, progression and resistance. We anticipate that the second year of this grant will be devoted to defining mechanisms underlying the role of Siah1/2 ubiquitin ligase in distinct subtypes of melanoma.

2. KEYWORDS

Siah1, Siah2, UPR, UBL, RNF5, RNF125, ubiquitin ligases, ER stress, JAK1, EGFR, melanoma, BRAF inhibitor resistance.

3. ACCOMPLISHMENTS

What were the major goals of the project?
The major goals of this project as stated in the approved SOW are as follows:

Specific Aim 1 – Establish the significance of the Siah2–hypoxia–ER stress regulatory axis in melanoma development and response to chemotherapy of a subset of melanoma
Major Task 1: Determine the unique characteristics of melanomas that harbor the SHE gene signature; months 1–5
Major Task 2: Examine the role of Siah1/2 in regulating the ER stress and hypoxia responses in melanomas that do and do not exhibit the SHE gene expression signature; months 3–12
Major Task 3: Determine the biological significance of the ATF4–Siah2 component of the SHE regulatory axis to key melanoma phenotypes in cultured and xenograft melanoma models; months 3–12
Milestone 1*: Will have refined the components along the ER stress and hypoxia pathways, contributing to melanoma growth and response to therapy in cluster of melanomas; months 1–18*

Specific Aim 2 – Determine the effect of inhibitors of the Siah2–ER stress axis on melanoma development and response to chemotherapy
Major Task 1: Determine the effect of Siah1/2 inhibitors in melanoma cultures *in vitro*; months 6–18
Major Task 2: Determine the effect of Siah1/2 inhibitors in melanoma xenografts *in vivo*; months 8–24

***Milestone 2**: Will have identified a novel approach to prevent and possibly overcome the resistance of melanoma to existing therapies*

Major Task 3: Determine the effect of Siah1/2 and ER stress inhibitors on melanoma development in genetic models, which recapitulates sun exposure of young age; months 8–24

***Milestone 3**: Will have defined the ability of ER stress and Siah inhibitors to impact sun-induced melanoma.*

What was accomplished under these goals?

Specific Aim 1. Establish the significance of the Siah2–hypoxia–ER stress regulatory axis in melanoma development and response to chemotherapy of a subset of melanoma
Major Task 1: Determine the unique characteristics of melanomas that harbor the SHE gene signature (months 1–5). ***This task has been completed**.*

We completed a comprehensive mapping of Siah1 and Siah2 in melanoma, which for the first time revealed that each of these ligases has a distinct network of downstream targets and is expressed in different clusters of melanoma tumors. As shown in **Figure 1**, we mapped the signaling networks that are associated with Siah1 and with Siah2—revealing that two ubiquitin ligases are responsible for the regulation of different cellular networks. As shown in **Figure 2**, we mapped melanomas that express Siah1 (shown) or Siah2 (not shown) allowing us to further explore the significance and biological implications of each of these ubiquitin ligases for respective cluster of melanomas.

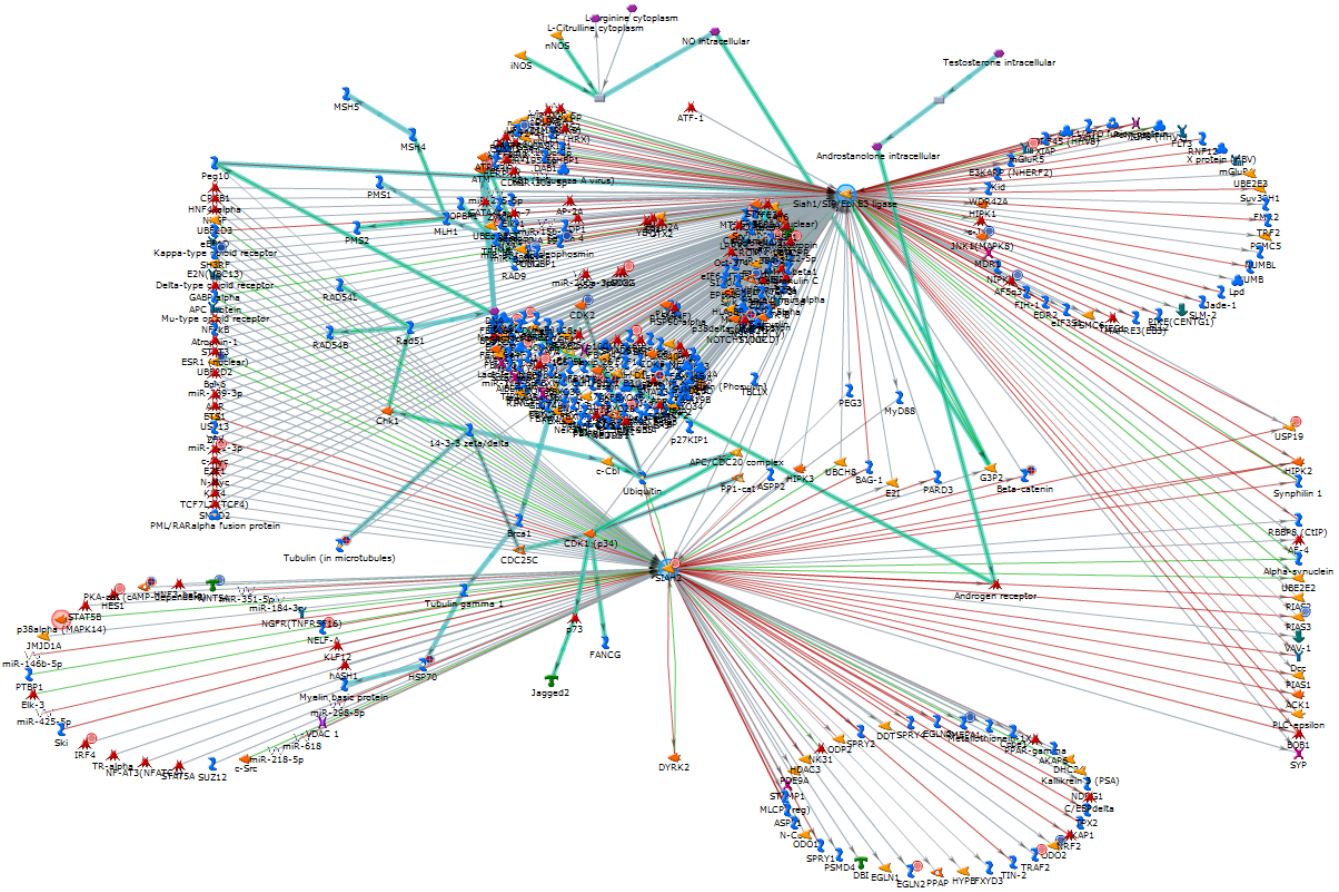


***Figure 1**. **Mapping Siah1 and Siah2 networks in melanoma**. The summary depicts exhaustive characterization of Siah1 and Siah2 regulatory pathways that were mapped based on collection of datasets that were studied. Each of these links, whether direct or secondary, has clear implications for the biological effect of these two ubiquitin ligases on key cellular networks in melanoma.*

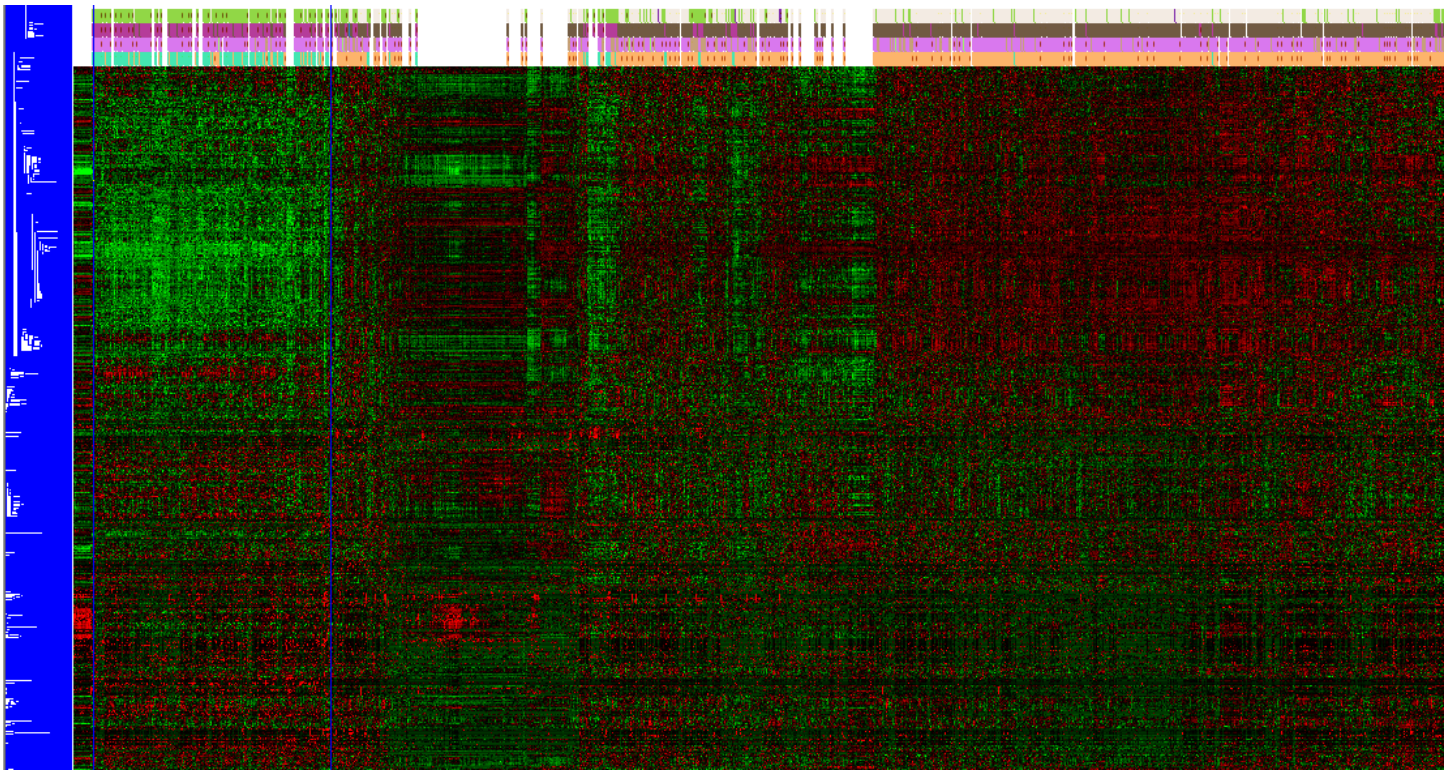


Figure 2. *Expression of Siah1 in melanoma establishes a clear subset that is positive for Siah1 expression (upper left green square). Similarly, a distinct subset of melanoma was identified as Siah2 expressing tumors.*

Major Task 2: Examine the role of Siah1/2 in regulating the ER stress and hypoxia responses in melanomas that do and do not exhibit the SHE gene expression signature (months 3–12). ***This task is 80% complete***.

We have begun to assess the role of Siah1 and Siah2 in the distinct cluster of melanomas. This led us to the unexpected discovery that Siah1 on its own consists of three splice variants, which are expressed to different degrees in melanoma. We thus needed to determine the differences among the three splice variants and identify which among them is the most significant in melanoma. Among these splice variants we identified Siah1L to be expressed in a way that is best linked to the resistant phenotype of melanoma.

Following a comprehensive set of experiments we identified that Siah1L is linked to the ER stress by its effect on both ATF4 and on PGC1α, master regulator of mitochondrial biogenesis. The focus on Siah1L has advanced our understanding of Siah in melanoma and revealed an unexpected layer in the regulation of ER stress and mitochondrial biology, which is currently being explored.

Major Task 3: Determine the biological significance of the ATF4–Siah2 component of the SHE regulatory axis to key melanoma phenotypes in cultured and xenograft melanoma models (months 3–12). ***This task is 20% complete***.

The discovery of Siah1 isoforms and the extensive studies we had to undertake to define their role in melanoma, relative to Siah2, has delayed the *in vivo* experiments outlined in this task.

Parallel studies with another ubiquitin ligase that is part of the ERAD, RNF5, led us to discover that it plays a key role in immune checkpoint control, which are reflected in the slow growth of tumors of the BRAF/PTEN/CDKN2A mutant genotypes in the RNF5 KO animals. **Figure 3** depicts the marked inhibition of tumor growth in the RNF5 KO mice, substantiating its key, although unknown, role in control of melanoma development. **Figure 4** depicts the significant increase in CD4 and CD8 positive

TIL and marked inhibition in the infiltration of effector T cells in tumors that are grown in the RNF5 KO mice, thereby pointing to its role in the control of immune checkpoint.

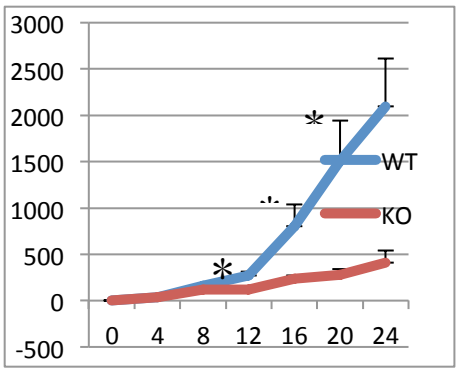
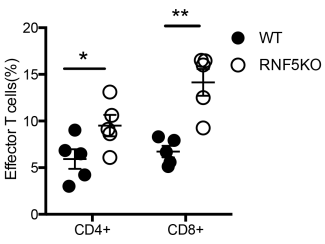


Figure 3. Attenuated growth of melanoma in RNF5 KO mice*. Melanoma tumors developed in B6 animals that carry V600E BRAF mutation, Pten deletion and Cdkn2a deletion were maintained in culture before injection (sc) into WT or RNF5 KO B6 mice. Growth of tumors was monitored over the indicated period and differences in tumor development were calculated for statistical power (P < 0.01 for 12, 16, 20 time points and P < 0.001 for the 24 day time point).*



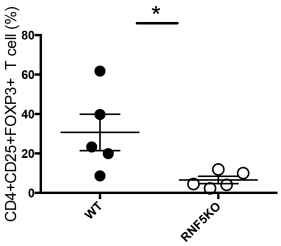


Figure 4. Increased CD4 and CD8 positive TIL in tumors grown in RNF5 KO mice*. FACS was performed at 16 and 24 days (shown is 24 day time point) after inoculation of tumor cells in the WT and RNF5 KO mice, and number of CD8 positive and CD4 positive cells was determined. Likewise the number of CD25-FOXP3-CD4 cells was determined, reflecting on the negative regulatory T cells whose presence has decreased in the tumors grown in the RNF5 KO mice.*

In a third project that was performed under the goal of defining ubiquitin ligase role in critical melanoma biology, we identified RNF125 as an ubiquitin ligase that is downregulated in tumors that developed resistance to therapy (BRAFi). We mapped the mechanism underlying RNF125 function in the resistant tumors and identified JAK1 as its substrate. Further, RNF125–JAK1 was found to affect expression of RTK including EGFR, PDGFR and AXL, which were shown to be important in melanoma resistance phenotypes. This study was published few months ago (*Cell Reports* 2015, see section 6), and is being considered for clinical trial, as we have shown that inhibition of JAK1 will help attenuate the resistance of melanoma to Vemurafenib.

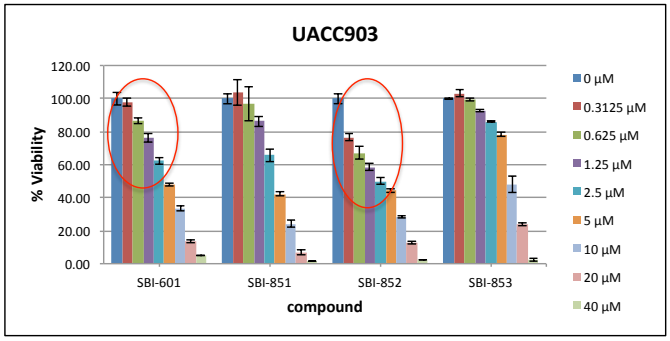
Milestone 1: Will have refined the components along the ER stress and hypoxia pathways, contributing to melanoma growth and response to therapy in cluster of melanomas; (months 1–18)

We have completed this milestone beyond original expectations as reflected in our current understanding of Siah1 and its isoforms, and Siah2, distinct function and respective melanoma cluster in which these are expressed. Added to this is our work on ER stress and RNF5, which identifies an unexpected role of this UBL in immune checkpoint control, and the discovery and characterization of RNF125 in melanoma resistance to vemurafenib.

Specific Aim 2. Determine the effect of inhibitors of the Siah2–ER stress axis on melanoma development and response to chemotherapy

Major Task 1: Determine the effect of Siah1/2 inhibitors in melanoma cultures ***in vitro*** (months 6–18). This task is 50% complete.

Substantial effort was devoted to develop a class of Siah inhibitors that would be amenable for *in vivo* evaluation, which is not a trivial task. We approached this by performing two parallel tasks. First, we advanced the development of a Siah inhibitory peptide, which we identified over the past couple of years and demonstrated its effectiveness in cultures of melanoma. We also developed the ability to administer a new class of peptides that were modified to enable *in vivo* delivery by IV injection and are currently testing them in tumor models in mice.



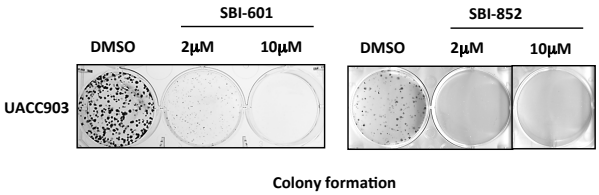
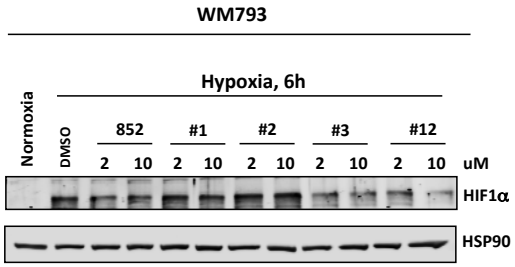


Figure 5. Characterization of Siah1/2 small molecule inhibitors. *A dose dependent evaluation of SBI-601 and SBI-852 performed on a series of melanoma cells demonstrates dose dependent inhibition of melanoma cell growth in 2D (upper) and 3D (lower) culture conditions (shown is representative line, UACC903).*

Additionally, we performed a high-throughput screen for small molecules that could affect Siah1/2 confirmation, and hence activity. Over 32,000 compounds were screened using a thermal shift assay, a sensitive method that identifies the change in the melting temperature of a protein, which is expected to occur upon tight association with a small molecule. Of 17 positive hits identified from this screen, a series of secondary and tertiary assays were carried out to verify activity of the select small molecules as Siah inhibitors. Three of the 17 compounds (#1, #3, and #12) were confirmed to exhibit inhibition of Siah ubiquitin ligases. As shown in Figure 6, each of these new compounds effectively inhibits the level of HIF1α, a surrogate marker for Siah1/2 activity.

Figure 6. Small molecule inhibitors of Siah1/2 attenuate the expression level of HIF1α. *Indicated concentrations of the Siah1/2 inhibitory small molecules were added to WM793 melanoma cells for 24 h before cells were harvested and assessed for the level of HIF1α protein. Since Siah1/2 is causing the degradation of prolyl hydroxylase 1/3, a negative regulator of HIF1α, inhibition of Siah2 is expected to increase the expression of PHD3 with a concomitant decrease in the level of HIF1α.*



Further, evaluation of these compounds in 3D growth, which were monitored for colony forming efficiency or their ability to grow on soft agar, identified compounds #1, #3, and #12 to exhibit the most significant effect (Figure 7 left panel). Likewise, assessment of these compounds in a soft agar growth of melanoma cells confirmed their effective inhibition of the cell's growth in semisolid medium (Figure 7 right panel). Notably, SBI-852 was used as a positive control in these assays, demonstrating its strong inhibition of Siah, and respectively, of HIF1α and CFE of melanoma cells (Figures 5 and 6). The differences in the degree of inhibition among the melanoma cell lines, and the distinct inhibitors is noted.

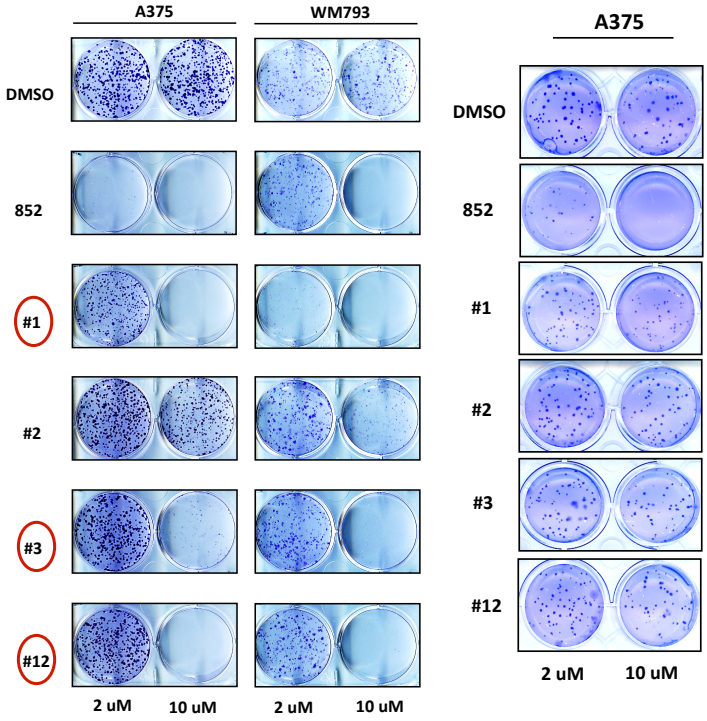


Figure 7. Select small molecules inhibitors of Siah1/2 effectively attenuate the 3D growth of melanoma cells. *Indicated melanoma cell lines were subjected to 3D growth in culture, where the effect of indicated small molecules was assessed. Five hundred cells plated were assessed 10 days later by crystal violet staining, demonstrating the dose-dependent inhibition of the melanoma growth by these inhibitors.*

Major Task 2: Determine the effect of Siah1/2 inhibitors in melanoma xenografts *in vivo* (months 8–24).

Experiments are expected to begin during the second year of funding.

Milestone 2: Will have identified a novel approach to prevent and possibly overcome the resistance of melanoma to existing therapies

We have accomplished this milestone by virtue of the development of first-in-class inhibitors for ubiquitin ligases, which reached evaluation *in vivo*, and by the advance in developing small molecule inhibitors for Siah using a state of the art approach. Characterization of both inhibitors during the second year will establish novel therapeutic modality for treatment of cancer, focused on melanoma.

Major Task 3: Determine the effect of Siah1/2 and ER stress inhibitors on melanoma development in genetic models, which recapitulates sun exposure of young age (months 8–24).

Will be carried out during the second year of funding

Milestone 3: Will have defined the ability of ER stress and Siah inhibitors to impact sun-induced melanoma

We expect to reach this milestone by the end of the second year of funding.

What opportunities for training and professional development has the project provided?

The following are the opportunities for training and professional development that this project has provided:

The Sanford Burnham Prebys Medical Discovery Institute (SBP) Office of Training & Academic Services oversees and coordinates an annual individual development planning (IDP) process for all postdocs at the Institute. The focus of the IDP process at SBP is the career goal of the postdoc; identification of what skills, knowledge, and accomplishments will be necessary for the postdoc to obtain a desired independent position following training; and identification of training and professional development opportunities that are available for the postdoc to obtain the necessary skills and knowledge. The SBP Office of Training & Academic Services provides guidance and advising to both postdocs and PIs throughout the postdoc's training with respect to developing IDPs and preparing for a successful transition to independence post-training. The SBP Office of Training & Academic Services also maintains webpages containing comprehensive resources on career path identification, career planning, and creating an IDP that can be utilized in conjunction with the formal annual IDP process. Dr. Yan Li, a postdoctoral trainee, is directly supported by this grant and works on the RNF5 project and is part of the active training program.

The SBP IDP process includes two components:

1. **First-Year IDP.** Within the first 3 months of beginning postdoctoral training at SBP, all postdocs receive and fill out an initial "planning and expectations" document to discuss with their PI. This document serves as the foundation for their postdoctoral IDP and is designed to facilitate discussion between the PI and new postdoc regarding goals and expectations for the first year of training, as well as stimulate initial discussions about long-term career goals and training plans.

2. **Postdoctoral IDP.** At the end of the first year of training SBP postdocs receive notification that it is time to update their IDP, and they receive the information they included in their first-year planning and expectations document in the form of a full IDP that they can update with their accomplishments over the past year and their goals for the coming year, mid-term future, and long-term future. Each subsequent year of their postdoctoral training, postdocs will receive notification and the previous year's IDP form to update and expand. The IDP forms are designed to build upon each previous year as well as provide a solid foundation from which a postdoc can easily build his or her CV/resume.

The SBP Office of Training & Academic Services also maintains webpages containing comprehensive resources on career path identification, career planning, and creating an IDP that can be utilized in conjunction with the formal annual IDP process.

How were the results disseminated to the communities of interest?

The results described in this report were presented at multiple international ubiquitin conferences, which were held in June 2015 in China and in September 2015 in Dobrovnik Croatia. The work was also presented in June 2015 in Iceland at a melanoma workshop. To the greater communities these results were disseminated through OSHER program at UCSD, in April 2015.

What do you plan to do during the next reporting period to accomplish the goals?

We expect to complete the tasks indicated in our proposal as detailed above. Specifically, we expect to define the role of Siah in select clusters of melanoma and use this information to stratify melanoma for treatment with the Siah inhibitors. We will define the melanomas based on the expression and activity of Siah1 vs. Siah2, further clustering melanoma based on ER stress related networks. We expect to proceed with the assessment of Siah2 inhibitors to the point we can evaluate them in genetic mouse and human PDX models *in vivo*. Along these we also expect to proceed with the characterization of the ER stress ubiquitin ligase RNF5 in context of its newly discovered role in immune checkpoint.

4. IMPACT

What was the impact on the development of the principal disciplines of the project?

The mapping of Siah ubiquitin ligases to distinct regulatory networks in a select set of melanomas enables a new level of refinement for these ubiquitin ligases.

What was the impact to other disciplines?

The identification of RNF5 – ER Stress UBL as player in immune checkpoint and the implications for melanoma points to the first UBL that has been mapped to the immune checkpoint control.

What was the impact on technology transfer?

Finding JAK1 as a novel target for treatment of melanoma with BRAF inhibitor resistance, based on our studies with RNF125 is evaluated for clinical trial in 2016.

What was the impact on society beyond science and technology?

Ability to develop peptides that specifically inhibit Siah ubiquitin ligase *in vivo* offers new paradigm for targeting ubiquitin ligases *in vivo*.

5. CHANGES/PROBLEMS

Changes in approach and reasons for change.

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them.

Nothing to report.

Changes that have a significant impact on expenditures.

Nothing to report.

6. PRODUCTS

Publications, conference papers, and presentations

Kim H, Frederick DT, Levesque MP, Cooper ZA, Feng Y, Krepler C, Brill L, Samuels Y, Hayward NK, Perlina A, Piris A, Zhang T, Halaban R, Herlyn MM, Brown KM, Wargo JA, Dummer R, Flaherty KT, Ronai ZA. Downregulation of the Ubiquitin Ligase RNF125 Underlies Resistance of Melanoma Cells to BRAF Inhibitors via JAK1 Deregulation. Cell Rep. 2015 Jun 9;11(9):1458-73.

Conference presentations

- UB workshop China, June 2015
- Melanoma workshop Croatia, June 2015
- Ubiquitin meeting Croatia, September 2015

Website(s) or other Internet site(s)

Nothing to report.

Technologies or techniques

Nothing to report.

Inventions, patent applications, and/or licenses

Development of Siah inhibitory peptide and small molecule inhibitor is expected to be filed as a patent.

Identifying JAK1 and its regulation by RNF125 in melanoma resistance was filed for protection.

Other products

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Ze'ev Ronai
Project Role:	Principal Investigator
Researcher Identifier:	
Nearest person month worked:	1
Contributions to Project:	PI; oversaw the development and progress of the project and coordinated the collaboration with a number of national and international groups.
Funding Support:	N/A

Name:	Hyungsoo Kim
Project Role:	Research Assistant Professor
Researcher Identifier:	
Nearest person month worked:	2
Contributions to Project:	Performed experiments related to Siah and RNF125.
Funding Support:	N/A

Name:	Yan Li
Project Role:	Postdoctoral Associate
Researcher Identifier:	
Nearest person month worked:	2
Contributions to Project:	Performed experiments related to RNF5.
Funding Support:	N/A

Name:	Alla Perlina
Project Role:	Bioinformatics Scientist
Researcher Identifier:	
Nearest person month worked:	1
Contributions to Project:	Developed the exhaustive bioinformatics-based assessment of Siah.
Funding Support:	N/A

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

The following is a description of active support changes since the submission of this grant:

Grants that have ended:

Melanoma Research Foundation (PI: Ronai, Z.) 10/01/13–09/30/15 0.0 calendar (0%)

$100,000

Understanding and Targeting ER Stress Pathways in Melanoma

Goals: This proposal focuses on characterizing the Siah2-ER stress pathway in melanoma, thereby contirubting to our understanding of the mechanisms underlying melanoma resistance, which in turn will lead to the development of novel therapies.

Specific Aims: (1) Characterize the deregulated ER stress response in a subset of BRAF WT melanomas. (2) Establish the significance of the Siah2-ER stress regulatory axis in melanoma development and response to chemotherapy. (3) Determine the effect of inhibitors of the Siah2-ER stress axis on melanoma development and response to chemotherapy.

Scientific Officer: Shelby Moneer
Melanoma Research Foundation
1411 K Street, NW Suite 800
Washington, DC 20005
Phone: (202) 347-9675
E-mail: smoneer@melanoma.org

5 R01 CA111515-09 (PI: Ronai, Z.) 07/01/10–04/30/15 1.2 calendar (10.0%)
NIH/NCI $194,203

Novel Insights in the Regulation of HIF1alpha Stability

Goals: The proposed studies will identify mechanisms underlying FoxA2 and Siah2 forms of prostate tumors. By characterizing the roles of FoxA2 and Siah2 these studies will provide insight into mechanisms underlying HIF activity and demonstrate the importance of these activities to prostate NE lesions/tumors that are known to be the more aggressive form of prostate cancer.

Specific Aims: (1) Determine the role of FoxA2, HIF-1α and Siah in human prostate tumor development and progression. (2) Characterize mechanisms underlying FoxA2 cooperation with HIF-1α. (3) Determine the role of select HIF-1α/FoxA2 regulated genes in prostate tumor development and progression. (4) Identify key domains required for FoxA2 association and cooperation with HIF-1α to determine whether inhibiting FoxA2/HIF-1α cooperation by use of select peptides blocks formation of NE phenotype and prostate tumors in mouse xenograft models using human CWR22Rv1 and LNCaP cells.

Grants Officer: Angela Urdaneta
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5 P01 CA128814-05 07/22/09–06/30/14
NIH/NCI $284,224
PI Project 1: Ronai, Z. 1.2 calendar (10%)
PI Core A: Ronai, Z. 1.2 calendar (10%)

Targeting Pten - An Upstream, Downstream and Offstream Approach

Goals: To study the role of Pten and related downstream signaling components in melanoma development and progression.

Specific Aims: (Program 1) Determine and characterize mechanisms underlying the regulation and function of the ubiquitin ligase Siah in melanoma tumorigenesis and metastasis. (Program 2) Define aspects of central carbon metabolism that are regulated by Pten/Akt and Siah, and assess whether these metabolic hubs are valid drug targets in Pten null melanoma tumors. (Program 3) Use structure-based drug design to develop, characterize and validate novel small molecule antagonists against Akt and Siah2.

Grants Officer: Angela Urdaneta
National Institutes of Health

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5 P30 CA30199-31 05/01/97–04/30/15
NIH/NCI $1,902,606
Associate Director: Ronai, Z. 1.2 calendar (10%)
Program Leader: Ronai, Z. 1.2 calendar (10%)
Cancer Center Support Grant
Goals: To provide director to the overall research mission pertaining to cancer research and to support shared service facilities, unique resources and recruitment of new staff members for the Institute's programs. This is an institutional grant, no funds from this grant are used to directly support Dr. Ronai's laboratory.
Grants Officer: Latosha Mathis
National Cancer Institute
6120 Executive Blvd.
Rockville, MD 20852
Phone: (301) 496-3177
E-mail: latosha.mathis@nih.gov

Melanoma Research Alliance (PI: Ronai, Z.) 05/01/11–04/30/14 0.12 calendar (1%)
$100,000
Combined Inhibition of NF-kappaB and AKT for Melanoma Treatment
Goals: This proposal focuses on the characterization of chemical compounds capable of inhibiting both AKT and NF-kappaB signaling pathways.
Specific Aims: (1) Validate effectiveness of BI-69-A11 in panel of 18 human melanoma cell lines, alone and in combination with B-Raf and MEK inhibitors. (2) Assess the effect of BI-69-A11 and B-Raf inhibitor on growth of human melanoma tumors transplants in mice. (3) Assess the effect of BI-69-A11 alone and with B-Raf inhibitor on development and metastasis of genetic mouse melanoma models.
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Pending grants that are now active:

1 R01 CA188372-01A1 (PI: Ronai, Z.) 04/01/15–03/31/20 1.2 calendar (10%)
NIH/NCI $309,569
Understanding and Targeting the Glutamine Carrier SLC1A5 in Breast Cancer
Goals: The major goal is to advance the understanding of Gln metabolism in BCa and provide the foundation for novel stratification methods and therapeutic modalities for BCa.
Specific Aims: (1) Identify RNF5-dependent and -independent transcriptional, translational, and post-translational events regulating SLC1A5/38A2 availability and activity in representative BCa cultures. (2) Establish the biological significance of SLC1A5/38A2 expression in BCa cells for cellular metabolism, mitochondrial dynamics and function, autophagy, growth, and response to therapy. (3) Using BCa tumor samples, circulating tumor cells and TMAs we will determine the relationship between BCa expression of SLC1A5/38A2 and RNF5, the response to treatment, and disease outcome.

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What other organizations were involved as partners?

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

Nothing to report.

9. APPENDIX

Nothing to report.